

1     Claims

2

3     1.    A human embryonic stem cell line  
4           characterised by at least one of the  
5           following:

6           i)    presence of the cell surface markers TRA-  
7                 1-60, GTCM2, and SSEA-4;

8           ii)   expression of *Oct-4*;

9           iii)  expression of *NANOG*;

10          iv)   expression of *REX-1*; and/or  
11                 expression of *TERT*.

12

13        2.    The human stem cell line as claimed in Claim  
14           1 having two or more of the characteristics  
15           i) to v).

16

17        3.    The human stem cell line as claimed in Claim  
18           2 having three or more of the characteristics  
19           i) to v).

20

21        4.    The human stem cell line as claimed in Claim  
22           3 having four of the characteristics i) to  
23           v).

24

25        5.    The human stem cell line as claimed in Claim  
26           4 having all of the characteristics i) to v).

27

28        6.    The stem cell line hES-NCL1 deposited at  
29           NIBSC under Accession No. P-05-001.

30

31        7.    An embryonic stem cell bank comprising a  
32           multiplicity of genetically distinct stem

1 cell lines as claimed in any one of Claims 1  
2 to 6.

3

4 8. A method of screening an agent for toxicity  
5 and/or for therapeutic efficacy, said method  
6 comprising:

- 7 i. exposing a stem cell line as claimed in  
8 any one of Claims 1 to 6 to said agent;  
9 ii. monitoring any alteration in viability  
10 and/or metabolism of said stem cells; and  
11 iii. determining any toxic or therapeutic  
12 effect of said agent.

13

14 9. A method of screening an agent for toxicity  
15 and/or for therapeutic efficacy, said method  
16 comprising:

- 17 i. exposing an embryonic stem cell bank as  
18 claimed in Claim 7 to said agent;  
19 ii. monitoring any alteration in viability  
20 and/or metabolism of said stem cells;  
21 and  
22 iii. determining any toxic or therapeutic  
23 effect of said agent.

24

25 10. A method of producing fibroblast-like cells,  
26 said method comprising:

- 27 i. providing a stem cell line as claimed in  
28 any one of Claims 1 to 6;  
29 ii. allowing cells of said stem cell line to  
30 differentiate into stem cell derived  
31 fibroblast-like cells.

32

- 1 11. The method of Claim 10 which is conducted  
2 without use of a specific stimulant for  
3 differentiation.  
4
- 5 12. The method as claimed in either one of Claims  
6 10 and 11 wherein the fibroblast-like cells  
7 are produced for a therapeutic purpose.  
8
- 9 13. A method of culturing cells wherein the  
10 fibroblast-like cells obtained as claimed in  
11 Claims 10 or 11 act as feeder cells or  
12 condition cell culture media used during  
13 culture of the cells.  
14
- 15 14. The method as claimed in Claim 13 wherein the  
16 cells being cultured are stem cells.  
17
- 18 15. A method of maintaining the viability of eggs  
19 prior to or during fertilisation, wherein the  
20 fibroblast-like cells obtained as claimed in  
21 Claims 10 or 11 act as feeder cells or  
22 condition cell culture media used during  
23 maintenance of the eggs.  
24
- 25 16. A method of culturing a blastocyst or embryo  
26 prior to implantation into a receptive  
27 female, wherein the fibroblast-like cells  
28 obtained as claimed in Claims 10 or 11 act as  
29 feeder cells or condition cell culture media  
30 used during culture of the blastocyst or  
31 embryo.  
32

- 1     17.    The fibroblast-like cell line hESCdF-NCL as  
2           deposited at ECACC under Accession No.  
3           04010601.  
4
- 5     18.    A method of culturing cells wherein hESCdF-  
6           NCL cells act as feeder cells or condition  
7           cell culture media used during culture of the  
8           cells.  
9
- 10    19.    The method as claimed in Claim 18 wherein the  
11           cells being cultured are stem cells.  
12
- 13    20.    A method of maintaining the viability of eggs  
14           prior to or during fertilisation, wherein  
15           hESCdF-NCL cells act as feeder cells or  
16           condition cell culture media used during  
17           maintenance of the eggs.  
18
- 19    21.    A method of culturing a blastocyst or embryo  
20           prior to implantation into a receptive  
21           female, wherein hESCdF-NCL cells act as  
22           feeder cells or condition cell culture media  
23           used during culture of the blastocyst or  
24           embryo.  
25
- 26    22.    A self-feeder system for the growth of  
27           undifferentiated stem cells, said system  
28           comprising:  
29           i.    culturing a stem cell line as claimed in  
30           any one of Claims 1 to 6; and  
31           ii.   and allowing some of the cells of said  
32           stem cell line to differentiate into

1 stem cell derived fibroblast-like cells  
2 whilst the remainder of the cells of  
3 said embryonic stem cell line remain in  
4 an undifferentiated pluripotent,  
5 multipotent or unipotent state, whereby  
6 said stem cell derived fibroblast-like  
7 cells act as autogeneic feeder cells for  
8 said stem cells.  
9

10 23. A method of culturing a blastocyst, said  
11 method comprising exposing said blastocyst  
12 for a period of at least 12 hours to Buffalo  
13 rat liver cells or to media conditioned by  
14 Buffalo rat liver cells.  
15

16 24. The method as claimed in Claim 23 wherein the  
17 period of exposure is at least 48 hours.  
18

19 25. The method as claimed in either one of Claims  
20 23 and 24 wherein the period of exposure of  
21 said blastocyst to said Buffalo rat liver  
22 cells or to media conditioned by said Buffalo  
23 rat liver cells immediately precedes  
24 extraction of ICM cells from the blastocyst.  
25

26 26. The method as claimed in any one of Claims 23  
27 to 25 wherein the media conditioned by  
28 Buffalo rat liver cells is produced by:  
29 i. culturing at least 75000 Buffalo rat  
30 liver cells/cm<sup>2</sup> in Glasgow medium for 24  
31 to 36 hours; and

- 1           ii.   recovering the media by removal of the  
2               cells.  
3
- 4   27.   The method as claimed in any one of Claims 23  
5           to 26 wherein the blastocyst can be cultured  
6           to day 8 after fertilisation and retain  
7           pluripotency.  
8
- 9   28.   The method as claimed in any one of Claims 23  
10          to 27 wherein said blastocyst is a primate  
11          blastocyst.  
12
- 13   29.   The method as claimed in Claim 28 wherein  
14          said blastocyst is a human blastocyst.  
15
- 16   30.   A method for culturing a blastocyst, as  
17          claimed in any one of Claims 23 to 29, said  
18          method comprising:  
19          i.     culturing said blastocyst from  
20               fertilisation in G1 media;  
21          ii.    transferring said blastocyst of step  
22                i) to G2.3 media and maintaining said  
23                blastocyst in the G2.3 media; and  
24          iii.   transferring said blastocyst of step  
25                ii) to cell culture media conditioned  
26                by Buffalo rat liver cells.  
27
- 28   31.   The method as claimed in Claim 30 wherein the  
29          blastocyst is cultured in the conditions of  
30          step i. for 1 to 3 days.  
31

- 1     32.    The method as claimed in either one of Claims  
2            30 and 31 wherein the blastocyst is cultured  
3            in the conditions of step ii. for 2 to 3  
4            days.  
5
- 6     33.    The method as claimed in any one of Claims 30  
7            to 32 wherein the blastocyst is cultured in  
8            the conditions of step iii. for 1 to 3 days.  
9
- 10    34.    The method as claimed in any one of Claims 30  
11           to 33 wherein the cell culture media is  
12           Dulbecco's modified Eagle's medium optionally  
13           supplemented with 15% (v/v) Glasgow medium  
14           and conditioned by Buffalo rat liver cells.  
15
- 16    35.    A method of *in vitro* fertilisation, said  
17           method comprising culturing a blastocyst as  
18           claimed in any one of Claims 23 to 34; and  
19           implanting said cultured blastocyst into a  
20           receptive female.  
21
- 22    36.    A method of producing an embryonic stem cell  
23           line, said method comprising:  
24           i.    culturing a blastocyst as claimed in any  
25                one of Claims 23 to 34; and  
26           ii.   extracting cells of the inner cell mass  
27                (ICM) from said blastocyst and culturing  
28                the cells to produce an embryonic stem  
29                cell line therefrom.  
30

- 1     37.    The method as claimed in Claim 36 wherein  
2            said stem cell line is a primate embryonic  
3            stem cell line.  
4
- 5     38.    The method as claimed in Claim 37 wherein  
6            said stem cell line is a non-human primate  
7            embryonic stem cell line.  
8
- 9     39.    The method as claimed in Claim 37 wherein  
10           said stem cell line is a human embryonic stem  
11           cell line.  
12
- 13    40.    The method as claimed in any one of Claims 36  
14           to 38 wherein said embryonic stem cell line  
15           is a pluripotent stem cell line.  
16
- 17    41.    A self-feeder system for the growth of  
18           undifferentiated stem cells, said system  
19           comprising:  
20           i.    culturing a blastocyst as claimed in  
21                Claims 23 to 34;  
22           ii.   extracting cells of the ICM from said  
23                blastocyst and culturing the cells to  
24                produce an embryonic stem cell line  
25                therefrom; and  
26           iii.   and allowing some of the cells of said  
27                embryonic stem cell line to differentiate  
28                into stem cell derived fibroblast-like  
29                cells whilst the remainder of the cells  
30                of said embryonic stem cell line remain  
31                in an undifferentiated pluripotent,  
32                multipotent or unipotent state, whereby



1           said stem cell derived fibroblast-like  
2           cells act as autogeneic feeder cells for  
3           said stem cells.

4

5    42.   An embryonic stem cell bank comprising a  
6           multiplicity of genetically distinct stem  
7           cell lines obtained by the method as claimed  
8           in any one of Claims 36 to 39.

9

10   43.   A method of producing fibroblast-like cells,  
11           said method comprising:  
12           i.   culturing a blastocyst as claimed in any  
13                one of Claims 23 to 34;  
14           ii.   extracting cells of the ICM from said  
15                blastocyst and culturing the cells to  
16                produce an embryonic stem cell line  
17                therefrom; and  
18           iii.   allowing cells of said embryonic stem  
19                cell line to differentiate into stem cell  
20                derived fibroblast-like cells.

21

22   44.   A method of culturing cells wherein the  
23           fibroblast-like cells obtained by the method  
24           of Claim 43 act as feeder cells or condition  
25           cell culture media used during culture of the  
26           cells.

27

28   45.   A method of maintaining the viability of eggs  
29           prior to or during fertilisation wherein the  
30           fibroblast-like cells obtained by the method  
31           of Claim 43 act as feeder cells or condition

1 cell culture media used during maintenance of  
2 the eggs.

3

4 46. A method of a blastocyst or embryo prior to  
5 implantation into a receptive female wherein  
6 the fibroblast-like cells obtained by the  
7 method of Claim 43 act as feeder cells or  
8 condition cell culture media used during  
9 culture of blastocyst or embryo.

10